REMARKS

The Examiner is thanked for the due consideration given the application. An Appendix containing two articles is attached to this paper. An IDS is being concurrently filed.

Claim 36 is pending in the application.

No new matter is believed to be added to the application by this amendment.

Rejection Under 35 USC §103(a)

Claim 36 has been rejected under 35 USC §102(b) as being anticipated by SAITO et al. (Proceedings of the American Association of Cancer Research Annual Meeting, March 2002, Vol. 43, pp. 257) in view of CONCINA et al. (J. Vasc. Res. 2000 May-June; 37(3): 202-8). This rejection is respectfully traversed.

As is set forth in claim 36, the present invention pertains to a process for preparing a monoclonal antibody directed against endothelial cells with an angiogenic phenotype that is capable of inhibiting angiogenesis. The antibody and endothelial cells include the properties of:

> "*said antibody binds to a surface of the endothelial cells with an angiogenic phenotype, and*
>
> *said antibody recognizes a unit present exclusively on the endothelial cells with an angiogenic phenotype,*
>
> *said endothelial cells having an angiogenic phenotype being obtained by culturing endothelial cells removed from an aorta in a medium containing a*

supplement consisting essentially of oestradiol and VEGF,

said endothelial cells with an angiogenic phenotype being such that:

said endothelial cells form tubes in presence of growth factor VEGF in a collagen gel,

said endothelial cells proliferate under action of VEGF,

said endothelial cells are protected from apoptosis by VEGF, and

said endothelial cells' expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype."

The process of claim 36 includes the steps of:

"immunizing an animal by injection of said endothelial cells with an angiogenic phenotype;

fusing myelomas of an animal and splenocytes of said animal in order to obtain hybridomas;

preparing a culture of said hybridomas;

cloning of said hybridomas and secreting antibodies against endothelial cells with an angiogenic phenotype; and

verifying that said antibodies inhibit said properties of said cells with an angiogenic phenotype."

SAITO et al. pertain to a method for producing monoclonal antibodies directed against epitopes expressed in the surface of tumor vasculature cells.

For this purpose, SAITO et al. teach the use of human umbilical vein endothelial cells (HUVEC) that are stimulated *in*

vitro with proangiogenic factors, i.e., basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF), and EEP, a growth factor obtained from new-born bovine brains added with murine Epidermal Growth Factor (EGF) and heparin. The cell treatment "activates" HUVEC cells, and they acquire an angiogenic phenotype.

Activated HUVEC are then used to immunize mice, in a generic method for producing hybridomas, to obtain monoclonal antibodies that specifically recognize epitopes expressed at the cell surface of tumoral activated endothelial cells, and that never interact with "normal" unactivated endothelial cells.

SAITO et al., by using a combination of growth factors that differs from the combination used in the present invention, i.e., ostradial and VEGF only, teach away from the present invention.

CONCINA et al. disclose that 17B-Esteradial (heareafter referred to as oestradial) has mitogenic effects on *in vitro* endothelial cells derived from fetal bovine aortic endothelial cells (FBAEC).

CONCINA et al. also teach that oestradiol treatment of FBAEC cells stimulates the gene expression, and secretion, of VEGF, in a dose dependant manner. As mentioned in Figure 3, a dose of 10^{-9} M of oestradial allows the expression, and secretion, of 0.2 ng/10^{6} cells of VEGF and a dose of 10^{-8} M of oestradiol allows the secretion of 0.3/10^{6} cells of VEGF.

Moreover, CONCINA et al. teach that it cannot be excluded that oestradiol might act on cells expressing VEGFR2 and thus be incorporated in active angiogenesis.

The Office Action asserts that one of skill in the art would have immunized mice with oestradial FBAEC treated cells, which express VEGF, to provide a method of producing antibodies reactive with tumor vasculature as taught by SAITO et al.

Moreover, the Office Action argues that HUVEC taught by SAITO et al and FBAEC taught by CONCINA et al. are both endothelial cells having an angiogenic phenotype, since they are both stimulated by angiogenic factors, and concludes that according to the *KSR v. Teleflex* decision (KSR, 550 U.S. at 416, 421), substituting a known element for another, to yield the known result, is obvious.

However, the combination of the teachings of SAITO et al. in view of CONCINA et al. should be considered to teach away, since the combination of the teachings of these two documents would lead the skilled artisan in direction divergent from the path that was taken by the applicant (see *In re Gurley*, 27 F.3d 551, 31 U.S.P.Q.2d 1130 (Fed. Cir.1994)).

The Office Action argues that HUVEC and FBAEC treated with angiogenic factors have to be considered both as endothelial cells with angiogenic phenotype.

At the filing date of the present application, the skilled person would know that many endothelial cells derived

from veins, arteries, or arterioles can be used to provide, after stimulation with angiogenic phenotype, endothelial cells with an angiogenic phenotype.

Therefore, the posited document combination can be extended to other endothelial cells derived from veins, arteries, arterioles, placenta, capillary, retina, etc.

At the time the invention was made, the skilled person knew that endothelial cells used as model for studying *in vitro* angiogenesis are, for instance, the following ones:

- ACEC, adrenal capillary endothelial cells,
- ATF, adipose tissue fragments,
- BAEC, bovine aortic endothelial cells,
- BCEC, bovine capillary endothelial cells,
- BREC, bovine retinal endothelial cells,
- CEB, blood-island-containing cystic embryoid bodies,
- CPAEC, calf pulmonary aortic endothelial cells,
- EB, embryoid bodies; ESC, embryonic stem cells,
- HCEC, human capillary endothelial cells,
- HDMEC, human dermal microvascular endothelial cells,
- HMMEC, human marrow microvascular endothelial cells,
- HPBV, human placental blood vessels,
- HUVEC, human umbilical vein endothelial cells,
- MMFP, mice microvessels fat pad,
- MTF, muscular tissue fragments,
- RAEx, rat aortic explants,

- RCEC, rat capillary endothelial cells, and
- RFMF, rat fat microvessels fragments.

(see for example Table 1 of VAILHE et al. (reproduced below), Laboratory Investigation, 2001, Vol. 81, No. 4, pp: 439-452, attached).

Table 1. In Vitro Models of Angiogenesis and Vasculogenesis[a]

Cells	Mean time required for the formation of CLS	Induction of morphogenesis[b]	Matrix	Spatial organization	Reference
BCEC, HCEC	3–6 wk	Tumor-cell conditioned medium	Gelatin	2-D	Folkman and Haudenschild, 1980
HUVEC	4–8 wk	S/C[b]	+/− Fibronectin, culture dish	2-D	Maciag et al. 1982
RAEx	1 wk	S	Clotted chick plasma	3-D	Nicosia et al. 1982
BAEC (Fetal and calf)	3 d–2 wk	S	Culture dish	2-D	Feder et al. 1983
RCEC	5 d	S	Amnionic membrane (basement surface)	2-D	Madri and Williams, 1983
RCEC	4 d	S	Type IV and V collagen (adsorbed)	2-D	Madri and Williams, 1983
BAEC	3–10 d	S	Type I collagen gel	3-D	Schor et al. 1983
BCEC	2–3 d	S	Cells sandwiched in Type I collagen gel	3-D	Montesano et al. 1983
ESC	12 d	S, CEB formation	Culture dish	3-D	Doetschman et al. 1985
MTF; ATF	3–12 d	S	Fibrin, Type I collagen gel	3-D	Montesano et al. 1985
BAEC, ACEC, HUVEC	1 d	S	Fibrin	2-D	Olander et al. 1985
BCEC	5–15 d	C	Type I collagen gel	3-D	Montesano et al, 1986
BCEC	2–3 d	Phorbol ester	Fibrin	3-D	Montesano et al. 1987
HUVEC, HDMEC	1 d	S	Matrigel	2-D	Kubota et al. 1988
BCEC	1–2 d	S/C[b]	Fibronectin, collagen IV, Gelatin	2-D	Ingber and Folkman, 1989b
RAEx	1 wk	S[b]	Fibrin and Type I collagen gel	3-D	Nicosia and Ottinetti, 1990a and b
BAEC	10–18 d	S	Type I collagen gel	2-D	Vernon et al. 1995
HUVEC	1 d	S	Cells sandwiched in fibrin I or II[c]	3-D	Chalupowicz et al, 1995
RFMF	4–6 d	S	Type I collagen gel	3-D	Hoying et al. 1996
CPAEC	2–7 d	S/C[b]	Microcarriers embedded in fibrin	3-D	Nehls and Herrmann, 1995a and b
HUVEC, BREC	1–2 d	S	Fibrin	2-D	Vailhé et al, 1996
ESC	11 d	S, EB formation	Semisolid methylcellulose	3-D	Vittet et al, 1996
HPBV	7–21 d	S	Fibrin	3-D	Brown et al, 1996
MMFP	2 wk	S/C[b]	Collagen gel	3-D	Arthur et al, 1998
BAEC	3–5 d	S/C[b]	Collagen gel	3-D	Vernon and Sage, 1999
BAEC, HUVEC	3 d	S	Type I collagen, fibrin	3-D	Korff and Augustin, 1999
HMMEC	21–50 d	S/C[b]	Type I collagen/fibronectin	2-D	Pelletier et al, 2000

ACEC, adrenal capillary endothelial cells; ATF, adipose tissue fragments; BAEC, bovine aortic endothelial cells; BCEC, bovine capillary endothelial cells; BREC, bovine retinal endothelial cells; CEB, blood-island-containing cystic embryoid bodies; CPAEC, calf pulmonary aortic endothelial cells; EB, embryoid bodies; ESC, embryonic stem cells; HCEC, human capillary endothelial cells; HDMEC, human dermal microvascular endothelial cells; HMMEC, human marrow microvascular endothelial cells; HPBV, human placental blood vessels; HUVEC, human umbilical vein endothelial cells; MMFP, mice microvessels fat pad; MTF, muscular tissue fragments; RAEx, rat aortic explants; RCEC, rat capillary endothelial cells; RFMF, rat fat microvessels fragments; CLS, capillary-like structures; C, cytokines; S, spontaneous.

[a] Adapted from Ingber and Folkman, 1989, with permission.
[b] See references for accurate details.
[c] Fibrin I (desA), fibrin which lacks fibrinopeptide A. Fibrin II (desAB), fibrin which lacks both fibrinopeptide A and B.

Table 1 of VAILHE et al. teaches that all the above mentioned cells can be used for *in vitro* angiogenesis and vasculogenesis. More precisely, Table 1 of VAILHE et a1. teaches that BAEC (from foetus or calf) are spontaneously able to induce morphogenesis when they are seeded on plate without cellular matrix component.

On the contrary, HUVEC cells and BREC cells are spontaneously able to induce morphogenesis when they are seeded on plate coated with fibrin.

Thus, VAILHE et al. teach that BREC cells and HUVEC cells are the closest ones, in terms of angiogenic potentialities, compared to FBAEC. Consequently, the skilled person would be motivated from the teaching of VAILHE et al., to replace HUVEC cells taught in the method by SAITO et al., by BREC cells instead of FBAEC cells.

At the time that the invention was made, one of skill in the art would have known that BREC cells stimulation by oestradiol induces VEGF gene expression, in a dose dependant manner, and also induces VEGF protein expression, as indicated respectively in Figure 4 and Figure 5 of SUZUMA et al., reproduced below (Investigative Ophtalmology & Visual Science, 1999, Vol 40, n. 9, pp: 2122-2129, attached).

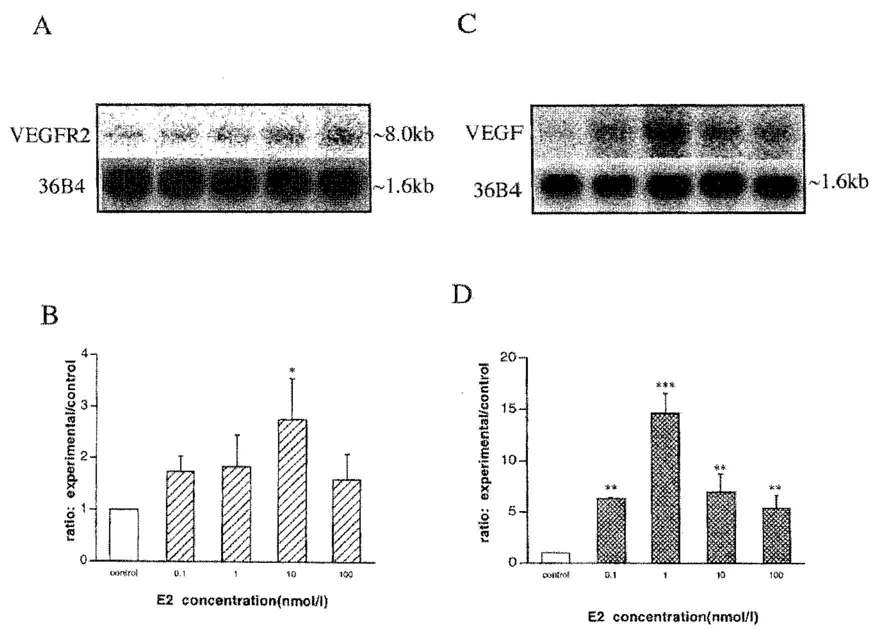

FIGURE 4. The effect of different doses of E_2 treatment on VEGFR2 (**A**, **B**) and VEGF (**C**, **D**) mRNA expression in BRECs. Subconfluent BRECs were cultured with various concentrations of E_2 for 9 hours. Cells were collected, and 20 μl RNA extracts were analyzed by Northern blot analysis with VEGFR2 mRNA probe. (**A**) Results from representative northern blot analysis are shown. (**B**) Densitometric analysis of the northern blot analysis data. $^*P < 0.05$. (**C**, **D**) The effect of different doses of E_2 treatment on VEGF mRNA expression in BRECs. Subconfluent BRECs were cultured with various concentrations of E_2 for 24 hours. Cells were collected, and 20 μl RNA extracts were analyzed by Northern blot analysis with VEGF mRNA probe. (**C**) Results from representative northern blot analysis are shown. (**D**) Densitometric analysis of the northern blot analysis data. $^{**}P < 0.01$; $^{***}P < 0.001$.

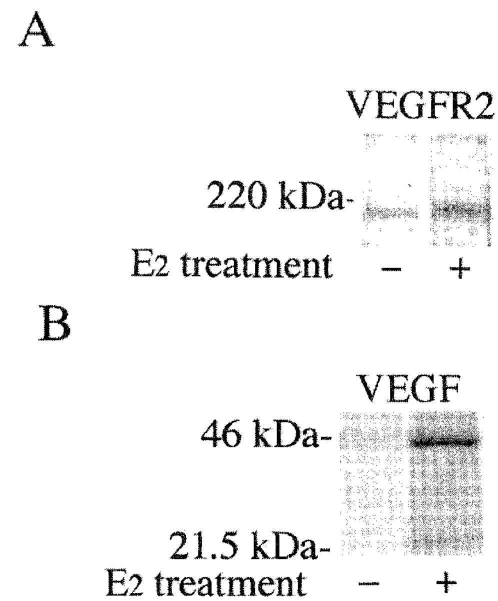

FIGURE 5. The effect of E_2 treatment on and VEGFR2 (A) and VEGF (B) protein expression in BRECs. Subconfluent BRECs were cultured with E_2-depleted medium or 10-nM E_2-replete medium. Cells were collected after 9 hours for VEGFR2 protein detection and after 24 hours for VEGF protein detection. Protein extracts were reacted with anti-VEGFR2 antibody or anti-VEGF antibody and immunoprecipitated with protein A-Sepharose beads. Each sample was electrophoresed and analyzed using a densitometer.

Therefore, from the teachings of SUZUMA et al. and the teachings of VAILHE et al., the skilled person would be motivated to use BREC cells of SUZUMA instead of FBAEC cells of CONCINA et al. to produce antibodies directed against tumor vasculature as disclosed in SAITO et al.

However, when substituting SAITO et al. cells by either SUZUMA et al. cells or CONCINA et al. cells, the skilled person would be led in direction divergent from the path that was taken by the applicant.

Indeed, pending claim 36 sets forth that ***"said endothelial cells having an angiogenic phenotype being obtained by culturing endothelial cells removed from an aorta in a medium***

consisting essentially of <u>oestradiol and VEGF</u>, said endothelial cells with an angiogenic phenotype being such that... <u>their expression of VEGFR-2 is increased 4-fold in comparison with cells with a non-angiogenic phenotype</u>".

SUZUMA et al. teach that oestradiol treated BREC cells are able to express VEGF growth factor, but also VEGFR·2 receptor. Moreover, at an oestradiol dosage of 10^{-8} M (i.e., 10 nM), the level of VEGFR-2 mRNA is increased 2.4 ± 0.3 times compared to untreated BREC cells. This increase corresponds to the maximal increased at this dosage (see page 2126, second column, first paragraph).

In contrast to the teachings of SUZUMA et al., CONCINA et al. never mention that the oestradiol stimulation enhances VEGFR-2 gene expression, or the level of said enhancement, if it exists.

Without such information, one of skill in the art would be led to prefer SUZUMA et al. cells instead of CONCINA et al. cells.

Consequently, at the time the invention was made, one of ordinary skill, having a knowledge of all the prior art, would be seek to provide a method for producing antibodies specifically interacting with endothelial cells having angionenic phenotype, by using SUZUMA et al. BREC cells stimulated with oestradiol, and secreting VEGF. However, since one of the main features of the

endothelial cells having an angiogenic phenotype used in this method is missing, the skilled person would be led in a direction divergent from the path that was taken by the applicant.

Indeed, since the cells used for the implementation of the method of SAITO et al. are different, the resulting monoclonal antibodies obtained by the method of SAITO et al. will be consequently different.

Attention should also be drawn to the fact that BREC cells are used in the application as filed, as negative control. It is discussed in the Examples Section of the specification that FN cells have an increase of the VEGFR-2 mRNA expression compared to F/O cells, whereas the expression of VEGFR-2 mRNA is identical in BREC/0 and BREC/V(Hutchings et al., 2002). The explanation is that Hutchings et al.'s BREC cells BREC/V have been stimulated, and thus activated, by the VEGF growth factor **ONLY**.

Indeed, the inventiveness of the present invention resides, in part, in the fact that for the first time endothelial cells with angiogenic phenotypes that have been stimulated by **only two exogenous** growth factor and hormones: VEGF and oestradiol. This particular growth factor and hormone stimulation confer to the cells specific new and inventive characteristics, allowing to obtain a new and inventive method for producing antibodies specifically directed against tumor vasculature.

Finally, if the skilled person has been motivated, for any reason, to combine the teachings of SAITO et al. and the

teachings of CONCINA et al., the skilled artisan would obtain a method for producing monoclonal antibodies directed against endothelial cells having angiogenic phenotype, but the endothelial cells having angiogenic phenotype obtained would never have **an expression of VEGFR-2 increased 4-fold in comparison with cells with a non-angiogenic phenotype**.

Indeed, one of the aims of the present invention is the importance of the **exogenous addition of VEGF** in the cell culture medium, which has a significantly higher efficacy than the VEGF secreted in response to oestradiol treatment.

Consequently, a person with ordinary skilled would never obtain the present invention, as claimed, from a knowledge of the teachings of SAITO et al. and the teaching of CONCINA et al. A *prima facie* case of unpatentability has thus not been made.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Request for Interview

If this amendment does not place the application in condition for allowance, the Examiner is requested to contact the applicant's representative, Robert E. Goozner, Ph.D., at 703-521-2297, in order to arrange an interview.

Conclusion

The rejections are believed to be overcome, obviated or rendered moot, and no issues remain. The Examiner is accordingly respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

Prior art of record but not utilized is believed to be non-pertinent to the instant claims.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX

The references cited in the accompanying Information Disclosure Statement:

- Suzuma et al.
- Vailhé et al.